Department of Neurology, Nanjing Hospital Affiliated to Nanjing Medical University, Nanjing, China

CYP2C19 polymorphism and antiplatelet effects of clopidogrel in Chinese stroke patients

YANG JIE, ZHAO HONG-DONG, TAN JIE, DING YUN-LONG, GU ZHI-QUN, ZOU JIAN-JUN

Received July 10, 2012, accepted August 10, 2012

Zou Jian-Jun, Department of Clinical Pharmacology, Nanjing Hospital Affiliated to Nanjing Medical University, Nanjing 210006, China
zoujianjun100@126.com

Pharmazie 68: 183–186 (2013) *doi: 10.1691/ph.2013.2703*

Recently published data indicate that CYP2C19*2 allele is the major determinant of metabolic bioactivation of clopidogrel and thereby variability of antiplatelet effect of clopidogrel in white or black patients undergoing elective coronary stent placement. The conclusion may not be fully generalized or extrapolated to the Chinese people due to significantly higher frequencies of the CYP2C19*2 or *3 variant alleles. We sought to investigate whether the CYP2C19*2 or *3 alleles affects platelet reactivity of clopidogrel in Chinese stroke patients. The study included 183 consecutive Chinese stroke patients after loading with clopidogrel 300 mg. Platelet function was assessed by adenosine diphosphate-induced (ADP 20 μmol/L) platelet aggregation and by light transmittance aggregometry (LTA) after seven 75-mg maintenance doses of clopidogrel before discharge. CYP2C19*2 or *3 genotypes were determined by time-of-flight mass spectrometer (MALDI/TOF-MS). In those patients who were carriers of 1 mutant allele (mutant heterozygotes, CYP2C19*1/*2 or *1/*3), ADP-induced maximum platelet aggregation (MPA) were significantly different compared with wild-type homozygous patients [37.2% (IQR, 19.6 to 50.5%) versus 23.6% (IQR, 14.0 to 35.4%), respectively; P = 0.002]. In addition, in the patients who were carriers of 2 mutant allele (mutant homozygotes, CYP2C19*2/*2, *2/*3 or *3/*3,), MPA were also significantly different compared with wild-type homozygous patients [35.7% (IQR, 21.0 to 78.1%) versus 23.6% (IQR, 14.0 to 35.4%, respectively; P = 0.039]. By multivariable linear regression, CYP2C19*2 or *3 loss-of-function alleles were independently associated with ADP-induced MPA measurements (partial $R^2 = 0.138$, P = 0.001). CYP2C19*2 or *3 allele does link to increased MPA and clopidogrel response.

1. Introduction

Current guidelines recommend a combination of clopidogrel and aspirin for the prevention of recurrent ischemic stroke (Aw and Sharma 2012; Fukuoka et al. 2011; Milionis et al. 2011). Despite this treatment, a substantial number of ischemic events with severe clinical consequences still occur (Simon et al. 2009). Several studies have shown a wide interindividual variability in the antiplatelet effects of clopidogrel, and patients with an inadequate antiplatelet response to clopidogrel are at increased risk for ischemic complications (Mega et al. 2009).

Clopidogrel, an inactive prodrug, requires two biotransformation steps mainly by the hepatic cytochrome P450 system to generate its active thiol metabolite, which targets and irreversibly binds to the platelet P2Y12 receptor and thereby inhibits adenosine diphosphate (ADP)-induced platelet activation and aggregation (Savi et al. 2000; Hollopeter et al. 2001). The highly polymorphic hepatic cytochrome P450 (CYP) system plays a key role in the conversion of clopidogrel into its active compound (Kazui et al. 2010). Several pharmacodynamic and outcome studies have demonstrated that CYP2C19*2 loss-of-function alleles are associated with a reduced antiplatelet effect of clopidogrel and a higher incidence of major cardiovascular events (Simon et al. 2009; Mega et al. 2009).

This finding, however, were performed in white or black patients. The conclusion may not be fully generalized or extrapolated to the Chinese people due to significantly higher frequencies of the CYP2C19*2 or *3 variant alleles (30%, 6.7%; Xie et al. 2011, 1997, 2001) in Chinese subjects than white (21.3%, 0.3%; Chang et al. 1996) and blacks (13.6%, 1.8%; Persson et al. 1996). To address the question of whether CYP2C19 polymorphism is associated with the antiplatelet response to clopidogrel in Chinese subjects, we investigated the impact of CYP2C19*2 or *3 alleles on the ADP-induced in 183 consecutive Chinese stroke patients treated with clopidogrel.

2. Investigations and results

2.1. CYP2C19 genotyping

The results of CYP2C19*2 (681G > A) and CYP2C19*3 (636G > A) genotyping are shown in Fig. 1. A cohort of 183 eligible stroke patients were divided into 3 groups according to their CYP2C19 genotype: wild-type homozygotes, CYP2C19 *1/*1, n = 87; mutant heterozygotes, CYP2C19 *1/*2 or *1/*3, n = 83; mutant homozygotes, CYP2C19 *2/*2, *2/*3 or *3/*3, n = 13. Baseline demographic and clinical characteristics of the study population according to their CYP2C19 genotype are summarized in Table 1. Their baseline characteristics of these stroke patients were well balanced between the three groups of genotypes of CYP2C19. Moreover, the distribution of the genetic variants did not deviate significantly from Hardy-Weinberg

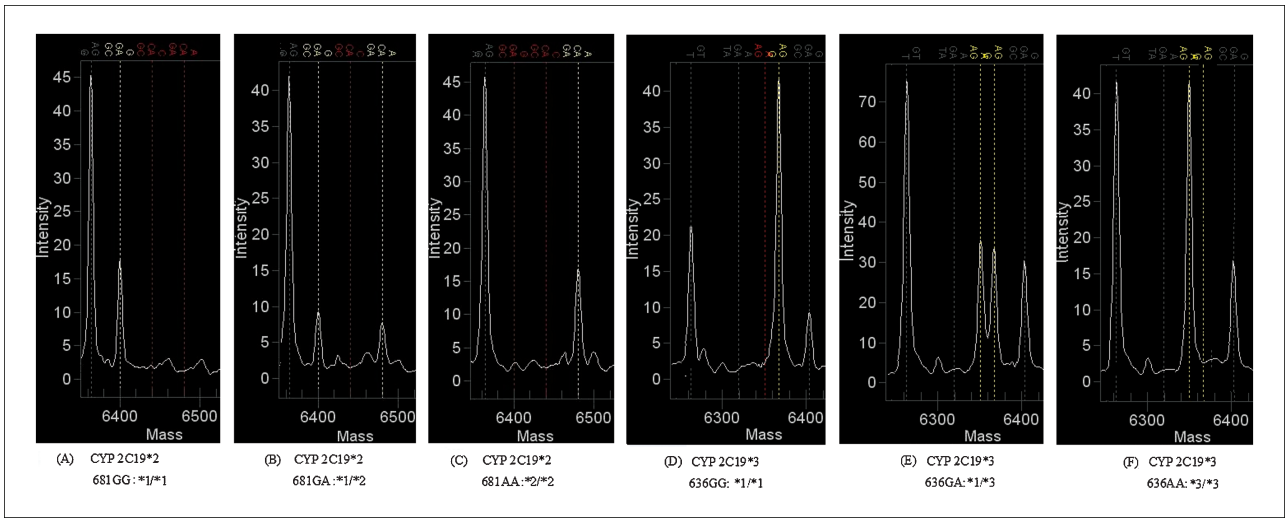

Fig. 1: Typical mass spectrograms for genotyping of CYP 2C19*2 [681GG: *1/*1 (A), 681GA:*1/*2 (B), 681AA:*2/*2 (C)] and CYP 2C19*3 [636GG: *1/*1 (D), 636GA:*1/*3 (E), 636AA:*3/*3 (F)]

Table 1: Demographic, baseline clinical and procedural characteristics of the patients undergoing PCI according to *CYP2C19* genotypes

	CYP2C19 *1/*1 (n = 87)	CYP 2C19 *1/*2, *1/*3 (n = 83)	CYP 2C19 *2/*2,*2/*3,*3/*3 (n = 13)	P value
Age, yrs	62.2 ± 8.5	63.1 ± 8.3	64.7 ± 8.9	0.568
Male (%)	64(73.6)	56(67.5)	12(92.3)	0.116
BMI, kg/m^2	24.6 ± 3.1	24.7 ± 2.7	24.2 ± 2.8	0.827
Hypertension	63(72.4)	60(72.3)	8(61.5)	0.720
Hyperlipidemia	46(52.9)	44(53.0)	6(46.2)	0.894
Diabetes mellitus	26(29.9)	19(22.9)	3(23.1)	0.564
Current smoking	26(29.9)	29(34.9)	5(38.5)	0.706
Statins	82(94.3)	78(94.0)	13(100.0)	0.467
ACE inhibitor	30(34.5)	37(44.6)	6(46.2)	0.360
Calcium-channel blocker	33(37.9)	37(44.6)	5(38.5)	0.666
Omeprazole	74(85.1)	73(88.0)	11(84.6)	0.844
Platelet count, × 10^9/L	209.9 ± 52.6	195.4 ± 59.6	213.7 ± 128.3	0.305
HDL-cholesterol, mmol/L	0.97 ± 0.23	1.04 ± 0.37	1.07 ± 0.31	0.258
LDL-cholesterol, mmol/L	2.56 ± 0.72	2.69 ± 0.79	2.76 ± 1.17	0.496
MPA (%)	26.9 ± 17.5	38.9 ± 24.3	47.6 ± 32.0	0.003

Values are n (%) or mean ± SD. BMI = body mass index; Statins, including atorvastatin, lovastatin, or simvastatin; ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; LDL = low-density lipoprotein, MPA = maximum platelet aggregation.

equilibrium ($X^2 = 1.089$; P = 0.25), the frequency of the *2 or * 3 allele of CYP2C19 was 29.8%.

2.2. MPA and CYP2C19 genotypes

The median value of ADP-induced MPA in the study population was 30.5% (IQR, 14.9 to 48.9%). ADP-induced MPA was significantly different between the 3 genotype groups (P = 0.003) (Table 1). As demonstrated in Fig. 2, in the 83 patients who were carriers of 1 mutant allele (CYP2C19*1/*2 or *1/*3), ADP-induced MPA were significantly different compared with wild-type homozygous (n = 87) patients [37.2% (IQR, 19.6 to 50.5%) versus 23.6% (IQR, 14.0 to 35.4%), respectively; P = 0.002]. In addition, in the 13 patients who were carriers of 2 mutant allele (CYP2C19*2/*2, *2/*3 or *3/*3), MPA were significantly different compared with wild-type homozygous (n = 87) patients [35.7% (IQR, 21.0 to 78.1%) versus 23.6% (IQR, 14.0 to 35.4%, respectively; P = 0.039]. In a multivariable linear regression model, CYP2C19*2 or *3 allele carriage was independently associated with ADP (20 μmol/L)-induced MPA (partial $R^2 = 0.138$, P = 0.001; Table 2), after adjustment with baseline clinical and demographic variables such as age, gender,

Table 2: Results of a multivariable linear regression model

Variable	β Coefficient Value	SE	P
CYP2C19 *2 or *3 allele carriage	11.39	2.96	0.001
Age	0.076	0.23	0.734
Male	–4.89	4.53	0.281
Body mass index	–0.97	0.65	0.141
Hyperlipidemia	–6.16	6.36	0.334
HDL-cholesterol	–1.65	6.20	0.790
LDL-cholesterol	1.12	2.69	0.677
Diabetes mellitus	–1.88	4.19	0.654
Hypertension	6.26	4.06	0.125
Use of statin	2.14	7.39	0.772
Use of omeprazole	3.21	5.19	0.538

Results of the multivariable linear regression model are shown for ADP-induced maximum platelet aggregation as the dependent variable.

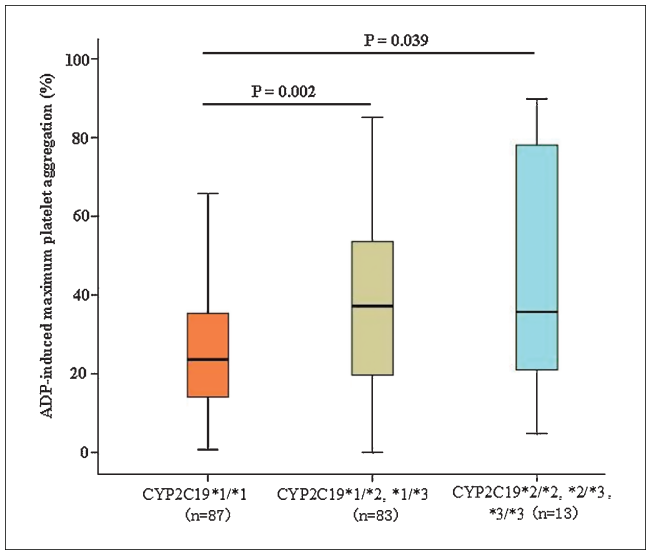

Fig. 2: CYP2C19*2 or *3 genotypes and maximum platelet aggregation. ADP-induced platelet aggregation (%) in relation to CYP2C19 genotypes (wild-type homozygotes, CYP2C19*1/*1; heterozygous, CYP2C19*1/*2, *1/*3; mutant homozygotes, CYP2C19*2/*2, *2/*3, *3/*3). Platelet aggregation values were compared across all genotype groups with the Kruskal-Wallis test and between groups with Mann-Whitney U test

diabetes, body mass index, HDL, LDL, hypertension, concurrent treatment with statin/omeprazole, suggesting that CYP2C19*2 or *3 allele may link to increased MPA.

3. Discussion

This study aimed to analyze the influence of genetic variations of CYP2C19*2 or *3 on the antiplatelet effects of clopidogrel in Chinese stroke patients. We found that CYP2C19*2 or *3 allelic variant has a significant impact on platelet function, resulting in significantly higher ADP-induced MPA than homozygous patients for the CYP2C19 wildtype. The carriers of CYP2C19*2 or *3 allelic variant exhibited a decreased response to clopidogrel. These findings were confirmed by a multivariable linear model that showed significant association of CYP2C19 genetic status with platelet function after clopidogrel (P = 0.001). This model confirmed that CYP2C19*2 or *3 loss-of-function allele(s) carrier status is the strongest variable significantly contributing to the variability in ADP (20 μmol/L)-induced MPA on clopidogrel, after adjustment with baseline clinical and demographic variables. Our observation regarding CYP2C19*2 is supported by the results of previous studies reported that carriage of the CYP2C19*2 allele is associated with the enhanced platelet function (Hulot et al. 2006; Harmsze et al. 2010; Jeong et al. 2010; Collet et al. 2009; Lee et al. 2011). Recent mechanistic investigations have demonstrated that the transcriptional activity and expression of CYP2C19 is significantly downregulated in the presence of the *2 allele (Harmsze et al. 2010).

To the best of our knowledge, this is the first study to report on the impact of the CYP2C19*2 or *3 allelic variant on both ADP-induced MPA in Chinese stroke patients treated with clopidogrel. The association of CYP2C19*3 genetic variants and the antiplatelet effects of clopidogrel is not explored in these studies (Simon et al. 2009; Mega et al. 2009; Hulot et al. 2006; Harmsze et al. 2010; Jeong et al. 2010; Collet et al. 2009; Lee et al. 2011; Harmsze et al. 2010) due to lower allele frequency of CYP2C19*3 in white (0.3%; Chang et al. 1996) and black (1.8%; Persson et al. 1996) than that in the Chinese population (6.7%; Xie et al. 2011, 1997, 2001).

These data suggest that routine genotyping of the CYP2C19*2 or *3 may help to identify patients who are at higher risk of developing ischemic events when clopidogrel is used as antiplatelet therapy to prevent ischemic events after stroke. Given the devastating consequences and poor prognosis of patients with ischemic events, great efforts should be made to further define a specific subgroup of patients in whom the risk of ischemic events is substantially increased. Because of increased risk for ischemic events in patients with high ADP-induced MPA values caused by the CYP2C19*2 or *3 allelic variant, we can establish a genetic risk factor in the form of the CYP2C19*2 or *3 allele or platelet function testing, identify those patients at high risk for ischemic events and define a therapeutic window for clopidogrel antiplatelet treatment, and those patients would benefit most from an alternative strategy.

The present study has its limitations that need to be discussed. First, we assessed the impact of only two single genetic and functionally relevant variants on ADP-induced MPA. The interaction of a number of genetic variants and their combined impact on MPA measures were not studied. Second, the active metabolite of clopidogrel in plasma was not measured due to its chemical instability and difficulty getting it as a chemical standard. Third, only one platelet function method was used in this study, although MPA measured by LTA is widely used to assess the functional status of platelets. Finally, the number of stroke patients was relatively small, which underscores the need for further studies to corroborate the present results.

In conclusion, we have shown that the carriage of the loss-of-function alleles CYP2C19*2 or *3 may have a significant impact on platelet function, resulting in significantly higher ADP-induced MPA, which would increase the risk on ischemic events. Personalized therapy targeting patients who carry these genetic variants might help to improve the clinical outcome of stroke patients.

4. Experimental

4.1. Subjects

The present study was conducted in Han Chinese patients with stroke. A total of 183 consecutive stroke patients (aged 18–75 years) after pretreatment with 300 mg of clopidogrel and aspirin were eligible for the inclusion criteria in a single-center, prospective observational cohort in the department of neurology of Nanjing First hospital in China; the inclusion period lasted from July 2010 until July 2011. The exclusion criteria were active bleeding and bleeding diathesis, platelet count $<100 \times 10^9$/L, severe renal or hepatic disorder, hematologic disorder, active malignancy, use of hormone replacement therapy or contraceptives, and premature clopidogrel or aspirin cessation or nonadherence.

4.2. Study protocol

The study was performed in accordance with the ethical principles of the Declaration of Helsinki and was approved by the ethics committee of Nanjing First Hospital, Nanjing Medical University. All patients gave written informed consent to study participation and blood sampling for genomic assays. The patients received a loading dose of 300 mg of clopidogrel (Plavix®, Hangzhou Sanofi-Aventis Minsheng Pharmaceuticals Co. Ltd., Hangzhou, China), All patients received dual anti-platelet therapy (aspirin and clopidogrel). Aspirin (100 mg/day, lifelong), and clopidogrel (75 mg/day, for 12 months) were administered. The predischarge samples were drawn in the morning on day 7 but before intake of the maintenance dose of clopidogrel using tubes containing 3.8% sodium-citrate (NanGeer Biomedical Co., Ltd, SiChuan, China). Blood samples for aggregation testing were processed within 2 h after blood collecting. Information on the patients was obtained from the practicing physicians or derived from hospital readmission records.

4.3. CYP2C19 Genotyping by time-of-flight mass spectrometer

Genomic DNA was extracted using commercially available QIAamp DNA™ Blood Mini Kit (Qiagen, Venlo, the Netherlands). Primers were obtained from Sangon Biotech (Shanghai, China). Genotyping was per-

formed in Shanghai Benegene Biotechnology Co., Ltd. (Shanghai, China) using the chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF, Jackson et al. 2000) from MassARRAY Compact System (Sequenom, San Diego, CA, USA). To verify correct sample handling, genotyping was repeated in 20% of the randomly selected patients for all variants tested. Repeated genotyping revealed the identical results, and the call rate for CYP2C19 was 100%, respectively.

4.4. MPA assay

MPA was the maximal amplitude of light transmission observed while residual platelet aggregation (RPA) was measured by LTA in native platelet-rich plasma (PRP) after addition of ADP (Sigma-Aldrich, Munich, Germany) at final concentrations of 20 μmol/L using a 4-channel LBY-NJ aggregometer (PuLiSheng, Beijing, China) (Bouman et al. 2010). The PRP was prepared by centrifugation of citrated venous blood at 150 g for 15 min, and platelet-poor plasma (PPP) by centrifugation at 1,500 g for 20 min. PRP was adjusted to 200–250 $\times$ 10^9 platelets/L by dilution with autologous PPP. Aggregation results were expressed as percentage of maximal light transmission using PPP from the same patient as reference (100% transmission). The coefficient of variation of our optical aggregometry assay was less than 10%. MPA was measured by the same laboratory staff unaware of patient's outcomes and genotyping results.

4.5. Statistical analysis

Frequencies of categorical variables were given as counts (percentages) and continuous variables either as mean-standard deviation or as median with interquartile range. Categorical values or possible deviations of the genotype distribution from the Hardy-Weinberg equilibrium were analyzed with Chi-square or Fisher's exact test, as appropriate. Continuous variables with a Gaussian distribution were compared by means of the unpaired 2-tailed t test or ANOVA for >2 groups, whereas continuous variables with a non-Gaussian distribution were compared by Kruskal-Wallis test or Mann-Whitney U test. The effect of CYP2C19 genotypes on antiplatelet effects of clopidogrel determined by ADP-induced aggregation was assessed by linear regression analysis. The percentage of variability of on-clopidogrel MPA that could attribute to the variability in independent variables was derived from partial X^2 calculated by a multivariable linear model. This model comprised ADP 20 μmol/L–induced MPA as the dependent variable and CYP2C19 genotype as well as all baseline clinical and demographic variables shown in Table 1. All statistical analyses were performed with SPSS 16.0 (SPSS Inc, Chicago, III, USA). A value of $P < 0.05$ in the 2-tailed test was considered as significant.

Acknowledgments: This work was supported by the National Scientific Foundation of China grants (No.81100859; No.30901830), the Jiangsu Education Agency (No.10KJB32001), and the Nanjing Health Bureau (No.QYK10137).

References

Aw D, Sharma JC (2012) Antiplatelets in secondary stroke prevention: should clopidogrel be the first choice? Postgrad Med J 88: 34–37.

Bouman HJ, Parlak E, van Werkum JW, Breet NJ, ten Cate H, Hackeng CM (2010) Which platelet function test is suitable to monitor clopidogrel responsiveness? A pharmacokinetic analysis on the active metabolite of clopidogrel. J Thromb Haemost 8: 482–488.

Chang M, Dahl ML, Tybring G, Götharson E, Bertilsson L (1996) Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: Comparision with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. Pharmacogenetics 5: 358–363.

Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J (2009) Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet 373: 309–317.

Fukuoka T, Furuya D, Takeda H, Dembo T, Nagoya H, Kato Y (2011) Evaluation of clopidogrel resistance in ischemic stroke patients. Intern Med 50: 31–35.

Harmsze AM, van Werkum JW, Ten Berg JM, Zwart B, Bouman HJ, Breet NJ (2010) CYP2C19*2 and CYP2C9*3 alleles are associated with stent thrombosis: a case–control study. Eur Heart J 31: 3046–3053.

Harmsze A, van Werkum JW, Bouman HJ, Ruven HJ, Breet NJ, Ten Berg JM (2010) Besides CYP2C19*2, the variant allele CYP2C9*3 is associated with higher on-clopidogrel platelet reactivity in patients on dual antiplatelet therapy undergoing elective coronary stent implantation. Pharmacogenet Genomics 20: 18–25.

Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature 409: 202–207.

Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C (2006) Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 108: 2244–2247.

Jeong YH, Kim IS, Park Y, Kang MK, Koh JS, Hwang SJ (2010) Carriage of cytochrome 2C19 polymorphism is associated with risk of high post-treatment platelet reactivity on high maintenance-dose clopidogrel of 150 mg/day: results of the ACCEL-DOUBLE (Accelerated Platelet Inhibition by a Double Dose of Clopidogrel According to Gene Polymorphism) study. JACC Cardiovasc Interv 3: 731–741.

Jackson PE, Scholl PF, Groopman JD (2000) Mass spectrometry for genotyping: an emerging tool for molecular medicine. Mol Med Today 6:271–276.

Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O (2010) Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos 38: 92–99.

Lee JB, Lee KA, Lee KY (2011) Cytochrome P450 2C19 polymorphism is associated with reduced clopidogrel response in cerebrovascular disease. Yonsei Med J 52: 734–738.

Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT (2009) Cytochrome P-450 polymorphisms and response to clopidogrel. N Engl J Med 360: 354–362.

Milionis HJ, Gerotziafas G, Kostapanos MS, Vemmou A, Zis P, Spengos K (2011) Clopidogrel vs. aspirin treatment on admission improves 5-year survival after a first-ever acute ischemic stroke. Data from the Athens Stroke Outcome Project.Arch Med Res 42: 443–450.

Persson I, Aklillu E, Rodrigues F, Bertilsson L, Ingelman-Sundberg M (1996) S-Mephenytoin hydroxylation phenotype and CYP2C19 genotype among E-thiopians. Pharmacogenetics 6: 521–526.

Savi P, Pereillo JM, Uzabiaga MF, Combalbert J, Picard C, Maffrand JP (2000) Identification and biological activity of the active metabolite of clopidogrel. Thromb Haemost 84: 891–896.

Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N (2009) Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 360: 363–375.

Xie HG, Zou JJ, Hu ZY, Zhang JJ, Ye F, Chen SL (2011) Individual variability in the disposition of and response to clopidogrel: pharmacogenomics and beyond. Pharmacol Ther 129: 267–289.

Xie HG (1997) Direct evidence for the higher frequency of CYP2C19 allelic heterozygotes in Chinese subjects than in white subjects. Clin Pharmacol Ther 62: 691–692.

Xie HG, Kim RB, Wood AJ, Stein CM (2001) Molecular basis of ethnic differences in drug disposition and response. Annu Rev Pharmacol Toxicol 41: 815–850.